J. mar. biol. Ass. India, **46** (1) : 80 - 86, Jan. - June, 2004

Effect of feed concentrations on larval growth, survival and development of *Holothuria (Theelothuria) spinifera* Theel

P.S. Asha

Research Centre of Central Marine Fisheries Research Institute, Tuticorin,Tamil Nadu- 628 001, India

Abstract

The results of the study to standardize the larval feeding regime, for the mass production of sea cucumber juveniles through hatchery system are presented. Auricularia larvae after 48 hours of fertilization obtained from the induced spawning of *Holothuria spinifera* were used for the experiment to ascertain the effect of feed concentration on larval quality, growth, survival and development. The flagellate *Isochrysis galbana* at 0,1,2,4,and 8 x 10^4 cells/ml were provided as the feed for a period of ten days. The growth rate of the larvae was more or less similar in 1 x 10^4 and 2 x 10^4 cells/ml. However the percentage of survival, rate of stomach growth, percentage of late auricularia developed and the occurrence of normal symmetrical shape indicated that 2 x 10^4 cells/ml as the optimum larval feed concentration for the species.

Key words: Effect of feed on sea cucumber larvae

Introduction

The availability or concentration of feed plays an important role in the larval growth and survival. Microalgae, especially the nanoplankters are being provided as feed for rearing the larvae of sea cucumbers. The algal concentrations of 2 to 4 x 10^4cells/ml were indicated as the optimum in larval rearing of *Holothuria scabra, H. atra* and *H.spinifera* and *Stichopus japonicus,* (James *et al.*, 1994; Battaglene,1999; Ramofafia *et al.*, 1995; Asha and Muthiah, 2002 and Ito, 1995). In none of these studies, the effects of various feed concentrations on the larval growth and survival were attempted. But Archer (1996) stated that at high algal concentration, the ingestion rate of larvae of *S. mollis* was got reduced. Rearing the larvae of *H.scabra* at concentrations of 1 to 8 x 10^4 cells/ml, Morgan (2001) also observed that high algal concentration inhibited the larval growth and development. Though the larvae of *H. spinifera* were successfully reared, the effect of algal concentrations on larval shape, growth and survival have not been attempted so far. Hence experiments were conducted with larvae of this species at different concentrations of *Isochrysis galbana* and the results are discussed below.

The author is thankful to Prof. (Dr.) Mohan Joseph Modayil, Director and Dr. M.Rajagopalan Head, FEM Division, CMFRI, Kochi for their interest and encouragements, to Dr. P.Muthiah, Prin-

cipal Scientist, for his guidance and also for correcting the manuscript, and to Sri. J.X. Rodrigo, Technical Officer, TRC of CMFRI for providing the micro algal feed.

Material and methods

Auricularia larvae, of 48 hours old and having an initial size 362.4± 8.4μm (Mean ± SE), were utilised for the experiments. They were obtained from a spawning experiment carried out in January 2002. The larvae were reared at $^1/_2$ ml in 3 litre plastic aquarium bowls containing sand-filtered seawater of salinity 34.8-35.5 ppt and temperature 28°C to 30°C. Seawater was completely changed on alternate days and only half the quantity on other days. While on complete water change, the larvae were retained in a 40μm sieve and transferred to 3 l-glass beaker. After through mixing, 1ml sub sample was taken in a counting chamber for estimating the survival rate. For ten larvae, the total length and stomach length were measured using a microscope fitted with a micrometer. Then the larvae were transferred to their respective bowls. The micro algae *I. galbana* was provided at concentrations of 1,2, 4, and 8x10^4 cells/ml as feed. No feed was given to the control larvae. The maintenance and mass culture of the algae were carried out following the serial dilution technique (Gopinathan, 1982). For each concentration, triplicates were maintained and the experiment was conducted up to 10th day, as the larvae metamorphosed into non-feeding doliolaria stage. At the end of the experiment, the larval stages in each treatment were also noted by observing hydrocoel, stomatocoel and lateral folds as observed by Smiley (1986) and Dautov and Kashienko (1995).

The mean size and number of the larvae from 4,6,8 and 10th day were recorded. The mean differences in the size and number of ten day old larvae from the initial values of two day old larvae were considered for each treatment in the one way analysis of variance (ANOVA). The differences between treatments were tested for significance by multiple comparisons using SPSS 7.5 programme.

Results and discussion

High survival rate of 73.3% was observed for the larvae fed with 2x10^{4}cells/ml of *I.galbana* followed by 58.9% for 4x10^4 cells/ml and 52.6% for 1x 10^{4}cells/ml on the 10th day. The survival rate of the larvae fed with 8x10^{4}cells/ml gradually decreased and came down to 25.7% on 10th day. The lowest survival of 6.3% was observed in the control (Table 1).

On the 4th day, the unfed larvae showed a growth rate of 83.6 μm/day, where as, it ranged from 90 to 158.5μm/day in other concentrations. On the 8th day, maximum growth rate (80.5μm/day) was observed for the larvae fed with 2x10^4 cells/ml, followed by 77.9 and 69.4μm/day in 4 and 1 x 10^4-cell concentrations respectively. On the 10th day, the larvae reared in 2x10^{4}cells/ml registered a growth rate of 59.5 ±1.9 μm/day and those reared in 1x 10^{4}cells/ml showed 59.1±1.9μm/day. The larvae fed with 4 and 8 x 10^{4}cells/ml showed a growth rate of 54.8±7.6 μm/day and 50.3±6.0μm/day respectively. The

Table 1. *Mean survival percentage (n=30) of the auricularia of* Holothuria spinifera *at 0, 1, 2, 4 and 8 x 10^4 cells/ml of* Isochrysis galbana *(mean ± S.E).*

Days	Survival rate (%)at different concentrations of *I.galbana* (mean ± S.E).				
	0x10^4	1 x10^4	2 x10^4	4 x10^4	8 x10^4
4	71.8±4.12	85.9± 4.81	88.5± 3.08	88.9± 1.44	80.8± 6.84
6	44.9± 1.64	80.5± 2.09	87.8± 2.62	73.4± 3.37	66.7± 5.39
8	21.1±1.09	70.1±1.04	75.5± 0.32	66.7± 2.22	48.0± 1.85
10	6.4± 1.22	52.6± 0.35	73.3± 0.42	58.9± 1.40	25.7± 1.11

least growth rate of 19.0±6.6 μm/day was observed among the control larvae (Table 2).

The highest mean stomach length increase of 128.4μm with a growth rate of 16.1μm/day was observed in the larvae fed with 2x10^4 cells/ml. In 1x10^4 cells/ml, the stomach length increase was 100.4μm with a rate of 12.6μm/day, whereas in 4 and 8x10^4cells/ml, the length of stomach increase was 77.7 and 70.7 μm with a growth rate of 9.7 and 8.8 μm/day respectively. The unfed larvae, due to the shrinkage of stomach showed a value of – 6.9 μm with a growth rate of -0.9μm/day (Table 3).

In the control, none of the larvae developed in to the late auricularia stage (Fig.1a). Seventy percentage of larvae attained late auricularia stage among the larvae fed with 1x10^4cells/ml concentrations (Fig.1b). On the 10th day, the larvae fed with 2x10^4 cells/ml had maximum percentage (80%) of late auricularia stage, 15% and 5% mid and early auricularia respectively (Fig.1c). In concentration of 4x10^4, 45% were late auricularia, 35% mid auricularia and 20% early auricularia.

Table 3. *Mean increase in length and growth rate of stomach (n=30) of auricularia larvae of* H. spinifera *at 0,1,2,4 and 8 x 10^4 cells/ml of* I. galbana *(mean ± S.E).*

Feed Concentrations (Cells/ml)	Length increase on 10th day (mean ± S.E). (μm)	Growth rate (mean ± S.E). (μm/day)
0x10^4	- 6.9± 1.4	- 0.9± 0.2
1x10^4	100.4± 1.9	12.7± 0.2
2x 10^4	128.4± 0.53	16.1± 0.1
4x10^4	77.7± 3.5	9.7± 0.4
8x10^4	70.7± 4.2	8.8± 0.53

Table 2. *Mean growth rate (n=30) of the auricularia of* Holothuria spinifera *at 0, 1, 2, 4 and 8 x 10^4 cells/ml of* Isochrysis galbana (mean ± S.E).

Days	Growth rate (μm/day)at different concentrations of *I.galbana* (mean ± S.E).				
	0x10^4	1 x10^4	2 x10^4	4 x10^4	8 x10^4
4	83.6± 4.6	158.5±1.3	152.2± 0.9	109.5 ±0.7	90.2± 1.2
6	52.6± 2.4	106.3±0.6	104.2±0.9	83.2 ±0.9	71.5± 1.4
8	18.8±1.9	69.4± 0.3	80.5± 0.7	77.9±1.7	57.4± 0.9
10	19.0± 0.4	59.1± 2.6	59.5±0.1	54.8± 2.3	50.3±2.5

Table 4. *ANOVA table on numbers of* H. spinifera *larvae surviving on 10th day at 0,1,2,4 and 8 x 10⁴cells/ml of* I. galbana (p<0.05).

One way ANOVA

Treatments	Sum of Squares	df	Mean Squares	F	Sig.
Between groups	1761626	4	440406.4	9.023	.002
Within groups	488084.0	10	48808.400		
Total	2249710	14			

(Fig.1d). Only one percentage of larvae at 8x10⁴cells/ml attained late auricularia stage and majority of them (65%) remained in the early auricularia stage itself (Fig.1e).

The differences in the mean survival rates in different microalgal concentrations by one way analysis of variance indicated high degree of significance (p<0.002) (Table 4). In the multiple comparison analysis, it was observed that the differences in the survival between control and larvae fed in 1,2 and 4 x 10^4 cells/ml were significant (p<0.05). Significant differences were also observed between larvae fed with 2x10^4 to 4x10^4 and 8 x10^4cells/ml and also between 1x 10^4 with 8x 10^4 cells /ml. At 8x10^4 cells/ml, no significant differences were noted ei-

Table 5. *Multiple comparisons on numbers of* H. spinifera *larvae surviving on 10th day at 0,1,2,4 and 8 x 10⁴cells/ml of* I. galbana *(p<0.05).*

Multiple comparisons

Dependent Variable : Concentrations
LSD

Between Concentrations	Mean Difference (I-J)	Std.Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
0 and 10.0	-693.667*	180.386	.003	-1095.591	-291.743
0 and 20.0	-1004.667*	180.386	.000	-1406.591	-602.743
0 and 40.0	-460.333*	180.386	.029	-862.257	-58.409
0 and 80.0	-290.333	180.386	.139	-692.257	111.591
10 and 20.0	-311.000	180.386	.115	-712.924	90.924
10 and 40.0	233.333	180.386	.225	-168.591	635.257
10 and 80.0	403.333*	180.386	.049	1.409	805.257
20 and 40.0	544.333*	180.386	.013	142.409	946.257
20 and 80.0	714.333*	180.386	.003	312.409	1116.257
40 and 80.0	170.000	180.386	.368	-231.924	571.924

* P<0.05.

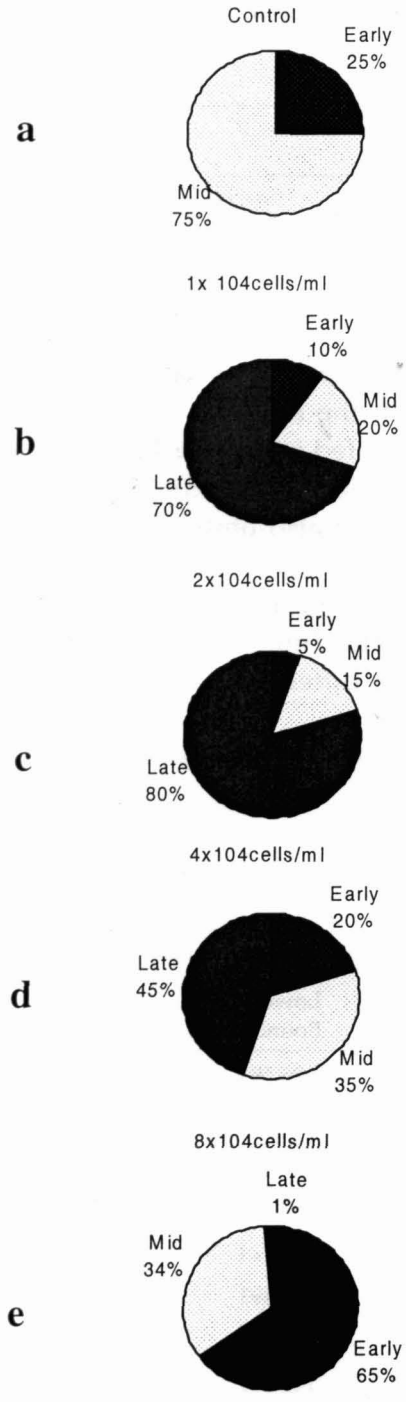

Fig. 1. *Proportions of auricularia (early, mid, and late) larvae of* Holothuria spinifera *in different feed concentrations.*

ther with control or with 4 x 10^4 cells/ml (Table 5). Similarly high survival percentage of *H .scabra* larvae was observed in 2x10^4 cells/ml than the control and other concentrations of 1,4 and 8 x10^4 cells/ml (Morgan, 2001).

Analysis of variance on the differences of mean growth rate among the various algal concentrations were highly significant ($p<0.05$) (Table 6). The multiple comparison carried out indicated that high significance ($p<0.05$) between the growth rate of the larvae fed with 2x10^4cells/ml, the control and other concentrations (Table 7). The growth rate of *H. spinifera* larvae at 1 and 2 x10^4cells/ml were more or less similar as Morgan (2001) reported for the larvae of *H.scabra.*

When compared to the larvae reared in other algal concentrations, those fed with 2x10^4cells/ml had a spherical shaped gut. Moreover, high significance ($t = 7.78$; $n = 4$; $p<0.001$) was observed in the mean stomach length increase at this concentration, which indicated a better larval quality as pointed out by Ito (1995) that the length and width of stomach are important indicators of quality of the larvae of *S. japonicus.* Similarly, Morgan (2001) noticed spherical gut in *H. scabra* fed with moderate amount of algae and the gut was contracted and variable in shape in higher algal concentrations

High percentage (80%) of late auricularia with normal symmetrical shape occurred on day 10 among the larvae in 2x 10^4cells/ml, whereas more asymmetry and irregularity in larval shape oc-

Table 6. *Anova table on growth rate of* H. spinifera *larvae on 10th day at 0,1,2,4 and 8 x 10⁴ cells/ml of* I. galbana *(P <0.05)*

One way ANOVA

Treatments	Sum of Squares	Df	Mean Squares	F	
Between groups	3431.463	4	857.866	29.600	.000
Within groups	289.822	10	28.982		
Total	3721.285	14			

curred in other concentrations as Morgan (2001) observed in *H.scabra* larvae. Well developed lipid spheres in late auricularia reared in 2x 10^4 cells/ml indicated the larval competency and readiness to metamorphosis as envisaged by Battaglene (1999) in *H.scabra*. The low survival, low growth rate and asymmetrical shape in larvae reared in 8x10^4cells/ml might have intercepted in the filtration, ingestion and digestion of auricularia as Morgan (2001) pointed out for *H.scabra* larvae. Archer (1996) similarly indicated that larvae of *Stichopus mollis* eventually stopped feeding when algal concentrations continuously exceeded 0.6 x10^4cells.

Table 7. *Multiple comparison on growth rate of* H.spinifera *larvae on 10th day at 0,1,2,4 and 8 x 10⁴ cells/ml of* I. galbana *(P<0.05) (Dependent variable: Concentrations)*

LSD

Between Concentrations	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
0 and 1.0	-40.043*	4.396	.000	-49.837	-30.249
0 and 2.0	-40.433*	4.396	.000	-50.227	-30.639
0 and 4.0	-35.807*	4.396	.000	-45.601	-26.013
0 and 8.0	-31.260*	4.396	.000	-41.05	-21.466
1 and 2.0	-.3900	4.396	.931	-10.1841	9.404
1 and 4.0	4.237	4.396	.358	-5.5574	14.031
1 and 8.0	8.783	4.396	.074	-1.018	18.578
2 and 4.0	4.626	4.396	.317	-5.1674	14.421
2 and 8.0	9.173	4.396	.063	-.6207	18.967
4 and 8.0	-4.547	4.396	.325	-5.2474	14.340

* P<0.05

Various concentrations of micro algae were provided in larval rearing of sea cucumbers. Optimal concentrations of 2 to 3x10⁴cells/ml for *H.scabra* larvae (James *et al.*, 1994) ; 10^4 to 10^5 cells/ml for *Actinopyga echinites* (Chen and Chian, 1990); 0.5 to 3x10⁴cells/ml for *Stichopus japonicus* (Ito,1995), 20 to 40,000 cells / ml^{-1} for *H. scabra* (Battaglene,1999) and 2 to 4 x 10^4 cells/ml for *H.spinifera* (Asha and Muthiah, 2002) have been reported. In the present study, the growth rate of *H.spinifera* was more or less similar in 1x10⁴cells/ml (59.1μm/day) and 2x10^4 cells/ml (59.5μm/day). But the highest rates of survival (73.3%), stomach growth (16μm/day), percentage of late auricularia developed (80%) and the occurrence of normal symmetrical shape of the larvae indicated that the 2x10⁴cells/ml of *I. galbana*, as the optimal algal feed concentration for the species.

References

Archer, J.E. 1996. Aspects of the reproductive and larval biology and ecology of the temperate holothurian *Stichopus mollis* (Hulton). *M.Sc Thesis*, University of Auckland, New Zealand, 189 pp.

Asha, P.S and P.Muthiah. 2002. Spawning and larval rearing of the sea cucumber *Holothuria (Theelothuria) spinifera* Theel. *SPC Beche-de-mer Information Bulletin*, **16**: 11-15.

Battaglene, S.C. 1999. Culture of tropical sea cucumbers for stock restoration and enhancement. *NAGA*, **22** (4): 4-10.

Chen, C.P and C.S. Chian. 1990. Short note on the larval development of the sea cucumber *Actinopyga echinites* (Echinodermata: Holothuroidea). *Bull. Inst. Zool, Academia Sinca.*, **29** (2): 127-133.

Dautov, S.S and S.D.Kashienko. 1995. Hyaline spheres in auricularia of *Stichopus japonicus. J. Invert. Rep. Develop.*, **27** : 61-64.

Gopinathan, C.P.1982. Methods of culturing phytoplankton. *In*: Manual of research method for fish and shell fish nutrition. *Spl. Publ.*, **8**: 113-118. Central Marine Fisheries Research Institute, Cochin.

Ito, S. 1995. Studies on the technological development of the mass production for sea cucumber juvenile, *Stichopus japonicus*. Saga Perfectural Sea Farming Centre, Japan; 87 pp.

James,D.B., A.D. Gandhi, N. Palaniswamy and J.X. Rodrigo.1994. Hatchery techniques and culture of sea cucumber *Holothuria scabra. Spl. Publ .*, **57**: 1-40. Central Marine Fisheries Research Institute, Cochin.

Morgan, A.D. 2001. The effect of food availability on early growth, development and survival of the sea cucumber *Holothuria scabra*. (Echinodermata: Holothuroidea). *SPC Beche-de-mer Information Bulletin.*, **14** : 6-12.

Ramofafia, C., M. Gervis and J. Bell. 1995. Spawning and early larval rearing of *Holothuria atra SPC Beche-de-mer Information Buletin.*, **7**: 2-7.

Smiley, S. 1986. Metamorphosis of *Stichopus californicus* (Echinodermata: Holothuroidea) and its phylogenetic implications . *Biol. Bull.*, **171**: 611-631.